Diquat (Reward®) Clinical Field Trials - INAD #10-969

Year 2007 Annual Summary Report on the Use of Diquat (Reward®) in Clinical Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U. S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

Summary

Diquat (Reward®) has been used effectively in the U. S. under compassionate INAD Exemption #10-969 to control mortality in a variety of fish species caused by common external fish bacterial pathogens. In calendar year 2007 (CY07) the efficacy of diquat (DQT) was evaluated in 32 disease trials involving approximately 2.6 million fish to control mortality in a variety of fish species caused by external columnaris or bacterial gill disease. Trials were conducted at five state fish culture facilities. Use of DQT under Protocol #10-969 allowed the investigator to administer therapeutic dosages of DQT to treat sick fish using one of the following two treatment options: 1) 2 - 18 mg/L for 1 - 4 h up to four times on consecutive or alternate days; or 2) 19 - 28 mg/L for 0.5 - 1 h up to three times on consecutive days. Overall, results indicated that treatments appeared effective in approximately 84% of the trials, ineffective in 3% of the trials, while 13% of the trials were characterized as inconclusive.

Introduction

Diseases of cultured fish often leads to severe losses of fish which can ultimately impact fish stocking programs and commercial fish farms. Such diseases can be caused by infections from a variety of fish pathogens. However, a few of these diseases, including bacterial gill disease (BGD), columnaris, and coldwater disease (CWD), appear to be the most prevalent.

Bacterial gill disease is one of the most serious diseases of intensively cultured fish, particularly young salmonids. If BGD is not diagnosed and treated early, significant mortality may occur in a very short period of time. Fish mortality is generally not a direct result of the infection, but is a consequence of the infection (i.e., structural changes in gill morphology). Stressors associated with intense fish culture may predispose fish to such an infection. Clinical signs of BGD have been well documented, and it is widely known that this disease can cause the rapid proliferation of gill epithelium and the production of excess mucus as the host responds defensively to the infection. This response can "smother" gills and result in severe fish losses if prompt measures are not taken. If BGD, which is horizontally transmitted, is not diagnosed and treated early, an epizootic may occur within a 24-h period (Bullock et al. 1990).

Columnaris - although *Flavobacterium branchiophilum* is the bacteria responsible for causing most outbreaks of BGD (Wakabayashi, et al., 1989; Ferguson et al., 1991), other gram-negative bacteria have also been implicated. These “other” bacteria include

pathogens such as *F. aquatile, F. psychrophilus* (causative agent of CWD), and *F. columnare* (causative agent of columnaris). Columnaris disease has been reported to cause significant mortality in a wide variety of fish (Post 1987), and is particularly devastating to cool and warm water species. Although the optimum temperature for the occurrence of columnaris disease is approximately 28 - 30^{o}C, epizootics often occur in cultured fishes at 10 - 17^{o}C. *F. columnare* typically first invades the skin of the head region, including the mouth, lips, cheeks, and gills and can result in necrosis of gill tissue. The pathogen also invades injuries or open wounds on the body of the fish. The type of lesions vary with the species of fish (Post 1987). Although *F. columnare* can routinely be detected externally in moribund fish when specimens are collected from the gills or open wounds of infected fish, the pathogen can also be cultured from kidney tissue of seriously infected fish. In such cases, columnaris disease is usually terminal within a relatively short time following bacteriemia (Post 1987).

Historically, several chemicals including benzalkonium chloride (available as Hyamine 1622 and 3500), diquat, and chloramine-T have been used to control mortality caused by BGD (Bullock et al. 1990) and other external flavobacteria. However, none of these chemicals have been approved by the FDA to control mortality in freshwater fish caused by such diseases. Although use of such chemicals does not guarantee success, INAD records support the use of chloramine-T and DQT to effectively control mortality in fish caused by external fish bacteria. The success of DQT as a chemotherapeutant that effectively controls mortality caused by external flavobacteria has been attributed to its characterization as a non-selective sanitizing agent that

effectively cleans up external fish surfaces, including skin and gills infested with bacteria. This report summarizes the use of DQT to control mortality in fish diagnosed with external bacterial diseases when used under INAD #10-969 during CY07.

Purpose of Report

The purpose of this report is to summarize the results of DQT field efficacy trials conducted under INAD #10-969 during CY07. We anticipate that data generated in these trials will be used to enhance data in the existing DQT database established from previous years, and will be considered in the "body of evidence" for the purpose of developing an appropriate label claim for the use of DQT in aquaculture.

Facilities, Materials, Treatment Procedures

1. Facilities

A total of five state fish culture facilities used DQT to control mortality in fish caused by BGD or external columnaris. Mean water temperature during all treatments was 70.9 °F, and water temperature for individual trials during treatments at the various testing facilities ranged from 42.0 - 83.1 °F.

2. Chemical material

REWARD® (a liquid DQT concentrate supplied by Syngenta Crop Protection, Inc., Greensboro, NC; 37.3% diquat bromide and 62.7% inerts) was the only brand of DQT used in CY07 trials, and remains the only brand of DQT that is allowed to be used under INAD #10-969 . This over-the-counter product contains 2 pounds diquat cation/gal as 3.73 pounds salt/gal.

3. Treatment Methods

Diquat (Reward®) treatments were administered by standing bath procedure. This procedure requires accurately measured amounts of liquid DQT to be pre-mixed in an appropriate amount of non-chlorinated water before administration.

Standing bath procedure - Water flow to the rearing unit is turned off, the pre-mixed chemical is added to the rearing unit, and contents of the rearing unit thoroughly mixed to ensure uniform DQT concentration throughout the rearing unit. Thorough mixing is essential to ensure there are no DQT "hot spots" and that regardless of a fishes position in the rearing unit, it is exposed to the target DQT dose. After the 0.5 - 4 hr treatment, water flow is turned on again to flush all DQT from the rearing unit.

4. Drug dosages

Diquat (Reward®) was used by Investigators at one or both of the following dosage regimens:

1. 4 - 18 mg/L for 1 hr - 4 hr
2. 20 - 28 mg/L for 1 hr

5. Number of DQT treatments administered per disease outbreak

According to the Study Protocol, Investigators were allowed to administer DQT on (1) 1 - 4 consecutive/alternating days when used at a dose of 2 - 18 mg/L or (2) 1 - 3 times on consecutive days at a dosage of 19 - 28 mg/L (approximately 66% of trials were conducted using these treatment regimens).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 34% of the trials) deviated from the protocol. In these trials, fish were treated at a dose of 10 - 18 mg/L DQT for durations that extended from 5 - 42 days. The Investigators noted that the deviations occurred primarily due to past use that supported the fact that high fish losses would result if DQT was not administered for periods exceeding what was allowed in the protocol. In addition, it has been shown that presence of high amounts of fine particles (e.g., uneaten feed, suspended organic solids) can irritate fish gills predisposing fish to columnaris and BGD infections. Such an event is typically associated with a period during the early grow-out phase when fish are fed fine particle sized feed,

or when water supplying the hatchery is only coarsely filtered and is not disinfected with either ultraviolet light or ozone.

Fish species that were used in efficacy trials in which these deviations occurred included: walleye, northern pike, musky, and channel catfish. However, it should be noted that because fish were treated at an early life-stage and will not be available for human consumption for at least 1-8 years, that there should be no concern regarding the withdrawal period.

Fish Species Treated and Fish Diseases Involved in CY07 Trials

1. Species and size of fish treated

A total of seven different non-salmonid fish species were treated during CY07. Mean length of treated fish was 3.4 in, and fish size ranged in length from 0.45 - 8.6 in.

Species treated included:

1. channel catfish *Ictalurus punctatus*
2. fathead minnow *Pimephales promelas*
3. largemouth bass *Micropterus salmoides*
4. muskellunge *Esox masquinongy*
5. northern pike *E. lucius*
6. redear sunfish *Lepomis macrolophus*

7. walleye *Stizostedion vitreum*

2. Diseases treated

The fish disease treated most frequently was characterized as external columnaris, which was treated for in 30 (94%) of the 32 trials; while BGD was treated for in two (6%) trials.

Data Collected

1. Pathologist's report

In the protocol, there is a request that a fish health biologist or qualified fishery biologist examine moribund and dead fish to try to determine the cause of death, and attach the fish health pathology report to the INAD data packet submitted to the AADAP Office following treatment. Fish health pathology reports can provide confirmation that there was a presumptive or definitive disease diagnosis for which treatment was recommended. Pathology reports were submitted for 28% of the trials submitted in CY07.

2. Mortality data

As stated in the Study Protocol, mortality data was to be collected for at least five days prior to treatment, during treatment, and for at least 14 d post-treatment. Investigators were strongly encouraged to collect mortality data on a daily basis. However, for a variety of reasons, not all requested mortality data was collected.

Reasons for an incomplete mortality record include: 1) splitting fish into additional rearing units to ease crowding and improve culture conditions, and 2) stocking early life stage fish shortly after final treatment.

Study Results - Discussion

1. General observations on the efficacy of DQT for the control of bacterial diseases in non-salmonid fish (Note: Table 1 provides a summary of all trials in which DQT treatments appeared effective; Table 2 provides a summary of all trials in which treatments were ineffective; Table 3 provides a summary of all trials in which treatments appeared inconclusive; Table 4 provides a summary of all treatment trials, including number of trials, number of fish treated, and treatment regimens used; and Tables 5a (trials sorted by study number) and 5b (trials sorted first by disease treated, second by whether or not treatments were effective, and lastly by fish species treated) provide a summary of all trials conducted during CY07 under INAD #10-969).

A. Efficacy of DQT When Used to Treat Columnaris

A total of 32 trials were conducted in which fish diagnosed with columnaris were treated with DQT at doses that ranged from 4 to 28 mg/L DQT for durations that ranged from 1 to 4 h. Fish were treated over a period that extended from 1 to 42 days (see Tables 1 - 3). Below is a list of the fish species that were treated for columnaris and the number of trials conducted using the specified treatment regimens:

channel catfish

1. Dose: 4 mg/L; Duration: 4 h; Treatment period: 1 day (5 trials)
2. Dose: 10 mg/L; Duration: 4 h; Treatment period: 3 - 11 days (2 trials)
3. Doses: 10 & 18 mg/L; Duration: 4 h; Treatment period: 3 - 20 days (1 trial)
4. Dose: 12 mg/L; Duration: 4 h; Treatment period: 1 day (1 trial)
5. Dose: 15 mg/L; Duration: 4 h; Treatment period: 2 - 38 days (4 trials)
6. Doses: 15 & 18 mg/L; Duration: 2 h; Treatment period: 1 - 2 days (1 trial)
7. Dose: 18 mg/L; Duration: 2 h; Treatment period: 1 - 3 days (2 trials)

fathead minnows

1. Dose: 12 mg/L; Duration: 4 h; Treatment period: 4 days (1 trial)

largemouth bass

1. Dose: 20 mg/L; Duration: 1 h; Treatment period: 3 days (3 trials)

muskellunge

1. Dose: 10 mg/L; Duration: 1 h; Treatment period: 42 days (1 trial)
1. Dose: 12 mg/L; Duration: 4 h; Treatment period: 2 days (1 trial)
1. Dose: 18 mg/L; Duration: 2 h; Treatment period: 3 - 16 days (2 trials)

redear sunfish

1. Dose: 28 mg/L; Duration: 1 h; Treatment period: 3 days (1 trial)

walleye

1. Dose: 10 mg/L: Duration: 1 h: Treatment period: 4 - 23 days (2 trials)
2. Doses: 10;12;15;18 mg/L; Duration: 1 h; Treatment period: 5 - 16 days (1 trial)
3. Dose: 12 & 15 mg/L; Duration: 4 h; Treatment period: 2 - 4 days (1 trial)
4. Doses: 12 & 18 mg/L; Duration: 2 h; Treatment period: 3 - 21 days (1 trial)

Results from 25 of the above-described trials indicated that treatments appeared effective, while one trial involving largemouth bass was ineffective, and four trials involving channel catfish were characterized as inconclusive.

B. Efficacy of DQT When Used to Treat Bacterial Gill Disease

Two trials were conducted in which northern pike were diagnosed with BGD and were treated with DQT at a dose of 10 mg/L for a 1 h duration. Fish were treated over a period that extended from 12 to 14 days (see Table 1). Both of these trials appeared to be effective.

3. Observed Toxicity

No toxicity or adverse effects relating to DQT treatments were reported in any of the trials.

Number of Treated Fish under Slaughter Authorization

Total number of treated fish during CY07 was 2,592,071. The total number of treated fish to count against the slaughter authorization dated August 9, 2002 (valid through October 30, 2007) is 19,800,905. The total number of treated fish to count against the current slaughter authorization dated October 31, 2007 is 93,474. No changes have occurred to the current DQT INAD #10-969 study protocol.

Facility Sign-up List

Please see "Table 6. Facilities and Names of Investigators" for facilities that signed-up to participate in the Diquat (Reward®) INAD #10-969 during CY07. Facilities not listed in the current Diquat (Reward®) INAD #10-969 study protocol have been highlighted.

Summary of Study Results

Diquat (Reward®) was used at doses ranging from 4 to 28 mg/L in 32 trials to control mortality in a variety of fish species caused by either columnaris or BGD. Fish were treated 1 - 42 times on consecutive or alternate days for durations that ranged from 1 to 4 h. Treatments were administered to seven different fish species, and treatment trials involved approximately 2.6 million fish. Mean length of fish treated during CY07 was 3.4 in (range, 0.45 - 8.6 in), and mean water temperature of all trials was 70.9°F (range, 42.0 - 83.1°F). Results from approximately 84% of trials indicated that DQT treatments appeared effective in controlling mortality, ineffective in 3% of the trials, and 13% of the trials were

characterized as inconclusive. Investigators reported no evidence of toxicity or adverse effects related to DQT treatment in any of the trials. Although data from these trials will be considered ancillary, trial results should provide useful corroborative data to support a future label claim for DQT. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #10-969. In future trials conducted under INAD #10-969, efforts will continue to be directed towards the generation of high quality data.

References

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Post, G.W. 1987. Textbook of fish health. Revised and expanded edition. TFH Publications, Inc., Ltd., Neptune City, New Jersey. 288 pp.

Wakabayashi, H, G.J. Huh and N. Kimura. 1989. Flavobacterium branchiophila sp. now., a causative agent of bacterial gill disease of freshwater fishes. International Journal of Systematic Bacteriology 39:213-216

Table 1. Summary of CY07 Diquat (Reward®) Field Efficacy Trial Results - Effective Treatments

Hatchery	Number of efficacious trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (°F)
Rathbun SFH/ Research Facility	2	5.0 - 6.2	CCF	39,715	Columnaris	1	4	4	77.0 - 81.4
Rathbun SFH/ Research Facility	2	5.3 - 7.3	CCF	142,619	Columnaris	3 - 11	10	4	46.0 - 67.0
Spirit Lake SFH	1	0.50	MUE	151,593	Columnaris & BGD	42	10	1	70.7
	2	0.75	NOP	306,580	BGD	12 - 14	10	1	64.7 - 66.2
New London SFH	1	0.45	WAE	96,700	Columnaris	4	10	1	66.5
Spirit Lake SFH	1	2.00	WAE	96,040	Columnaris & BGD	23	10	1	73.2
Rathbun SFH/ Research Facility	1	2.55	WAE	312,334	Columnaris	5 - 16	10;12;15; 18	1	72.0
Rathbun SFH/ Research Facility	1	5.30	CCF	127,286	Columnaris	3 - 20	10 & 18	4	63.0
Rathbun SFH/ Research Facility	1	1.70	CCF	139,940	Columnaris	1	12	4	83.1
	1	2.00	FHM	300,000	Columnaris	4	12	4	63.0
	1	8.60	MUE	8,238	Columnaris	2	12	4	64.0
Rathbun SFH/ Research Facility	1	3.40	WAE	225,008	Columnaris	2 & 4	12 & 15	4	74.0
Rathbun SFH/ Research Facility	1	1.00	WAE	72,000	Columnaris	3 - 21	12 & 18	2	73.4

Table 1. Summary of CY07 Diquat (Reward®) Field Efficacy Trial Results - Effective Treatments - cont.

Hatchery	Number of efficacious trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (°F)
Rathbun SFH/ Research Facility	3	2.5 - 7.8	CCF	189,826	Columnaris	2 - 36	15	4	42.0 - 79.0
Rathbun SFH/ Research Facility	1	4.50	CCF	3,875	Columnaris	1 - 2	15 & 18	2	77.5
Rathbun SFH/ Research Facility	2	3.0 - 4.5	CCF	7,971	Columnaris	1 - 3	18	2	79.1 - 80.5
	2	3.0 - 4.5	MUE	8,578	Columnaris	3 - 16	18	2	74.5 - 80.0
Richloam SFH	2	1.7 - 2.0	LMB	163,000	Columnaris	3	20	1	70.0 - 73.0
Jake Wolf Memorial SFH	1	1.50	RSF	30,500	Columnaris	3	28	1	75.0

Table 2. Summary of CY07 Diquat (Reward®) Field Efficacy Trial Results - Ineffective Treatments

Hatchery	Number of ineffective trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (°F)
Richloam SFH	1	1.70	LMB	66,000	Columnaris	3	20	1	70.0

Table 3. Summary of CY07 Diquat (Reward®) Field Efficacy Trial Results - Inconclusive Treatments

Hatchery	Number of inconclusive trials	Fish Size (in.)	Fish Species	Number of Fish	Disease	Number of treatment days	Dose (mg/L)	Duration (hrs)	Temp. (°F)
Rathbun SFH/ Research Facility	3	5.00	CCF	4,200	Columnaris	1	4	4	81.4
Rathbun SFH/ Research Facility	1	2.20	CCF	100,068	Columnaris	38	15	4	78.0

Table 4. Summary of Number of Treated Fish, Number of Treatment Trials, Treatment Regimens Used, and Fish Species Treated during CY07 Diquat (Reward®) Field Efficacy Trials

Total Number of Fish Treated:	**2,595,071**	
Number of fish treated in effective trials	2,421,803	
Number of fish treated in ineffective trials	66,000	
Number of fish treated in inconclusive trials	104,268	
Total Number of Trials:		**32**
Number of trials in which treatments were effective		27
Number of trials in which treatments were ineffective		1
Number of trials in which treatment results were inconclusive		4

Treatment Regimes and Frequency Used:

4 - 18 mg/L for 1 - 4 hr; 1 - 42 days	28 trials
20 - 28 mg/L for 1 hr; 3 days	4 trials

Treatment Water Temperature (°F):

Temperature Range	42.0 - 83.1
Mean Temperature	70.9

Size of Treated Fish (in.):

Fish Size Range	0.45 - 8.6
Mean Fish Size	3.4

Species Treated:

channel catfish *Ictalurus punctatus*
fathead minnow *Pimephales promelas*
largemouth bass *Micropterus salmoides*
muskellunge *Esox masquinongy*
northern pike *E. lucius*
redear sunfish *Lepomis macrolophus*
walleye *Stizostedion vitreum*